

EDITORIAL COMMENT

Nitroglycerin Hits the Nerve

Role for Mitochondrial Aldehyde Dehydrogenase?*

Ulrich Hink, MD,
Mohamed-Saiel S. Alhamdani, MD,
Thomas Münzel, MD, FAHA
Mainz, Germany

Organic nitrates are still widely used for the treatment of acute and chronic angina and congestive heart failure. When given acutely, their effectiveness is indisputable; in contrast, their long-term efficacy is limited because of serious side effects such as the development of tolerance and endothelial dysfunction. Results of a large observational study even indicate that nitrates had negative effects on the prognosis in patients who had experienced a myocardial infarction (1). Within the last 5 to 6 years, increasing evidence has supported a crucial role of the mitochondrial aldehyde dehydrogenase (ALDH-2) in the bioactivation process of nitroglycerin (GTN) (2). Nevertheless, the precise contribution of GTN-stimulated releases of other vasoactive substances such as prostacyclin and the neuropeptide calcitonin gene-related peptide (CGRP) remains obscure.

See page 953

ALDH-2 and GTN Bioactivation

Nitroglycerin exerts its beneficial, anti-ischemic effects via vasodilation of large coronary arteries, collateral vessels, and venous capacitance vessels while minimally affecting arteriolar tone (3). Nitroglycerin activates the soluble guanylyl cyclase (sGC), leading to cyclic guanosine 3',5'-monophosphate (cGMP) formation, which activates cGMP-dependent protein kinase (cGK-I). The cGK-I in turn causes vasorelaxation via phosphorylation of several proteins that regulate intracellular Ca^{2+} mobilization (4).

In 2002, Chen et al. (2) proposed that ALDH-2 is the predominant bioactivating enzyme for GTN. The investigators convincingly showed that ALDH-2, a mitochondrial enzyme, can act as a GTN-reductase to form 1,2-glyceryl dinitrate and a nitrite intermediate (NO_x), leading to cGMP

formation, and subsequently to vasodilation (2) (Fig. 1). The critical role of the ALDH-2 in GTN-induced vasodilation was further substantiated by studies in humans with a Glu504Lys ALDH-2-polymorphism. This polymorphism is characterized by a functional loss of ALDH-2 activity and a markedly reduced GTN bioactivation and vasodilatory capacity of GTN (5).

Calcitonin Gene-Related Peptide (CGRP) Contributes to GTN-Induced Vasodilation and Inhibition of Platelet Aggregation

Wei et al. (6) were the first to report in 1992 that nitrovasodilators such as GTN and sodium nitroprusside stimulate sensory nerves to release CGRP, which in turn relaxes cerebral vascular smooth muscle in cats by activating guanylate cyclase. The investigators suggested a role of CGRP in GTN-induced vasodilation and headache (6). The role of CGRP as a mediator of GTN effects was further substantiated by the observation that pre-incubation of rat aorta with GTN increases the release of CGRP (7), an effect that was blunted by the specific competitive CGRP receptor antagonist CGRP_{8-37} and by capsaicin, which desensitizes sensory nerves by transmitter depletion (8). Experimental studies showed that GTN-induced blood pressure decreases in rats were paralleled by increases in plasma concentrations of CGRP (9). In addition, GTN-induced inhibition of whole blood aggregation was partly inhibited by CGRP_{8-37} (10), suggesting that CGRP is also, at least partially, involved in the antiaggregatory effects of GTN.

Where Is CGRP Coming From?

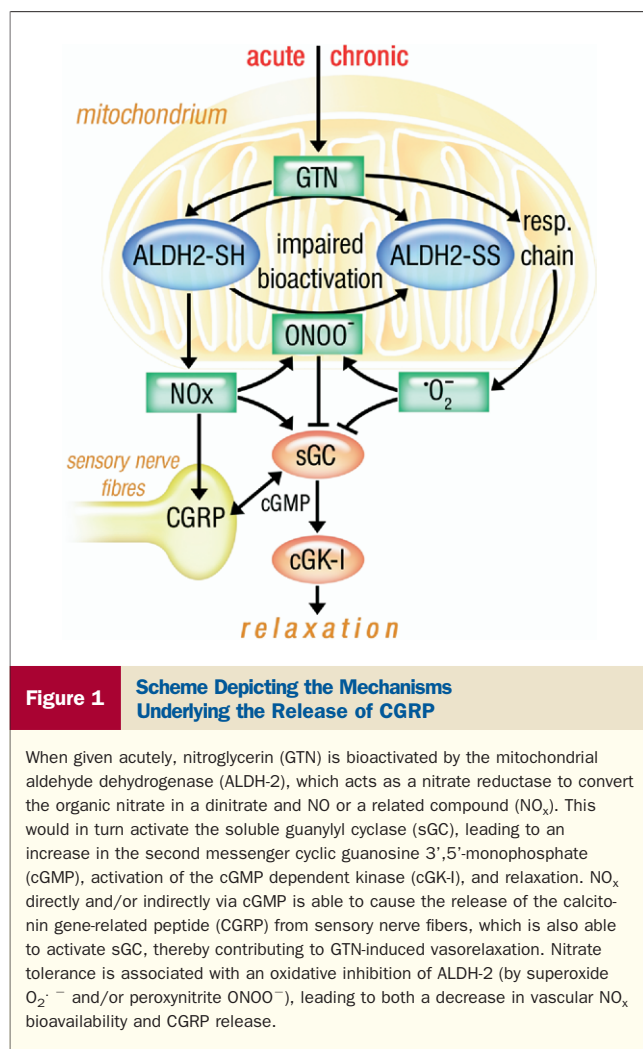
The 37-amino-acid vasoactive neuropeptide CGRP and other neuropeptides, like tachykinins, are coexpressed in primary afferent neurons that belong to the nonadrenergic noncholinergic (NANC) nervous system, which is involved in the sensation of pain (11). Furthermore, NANC neurons forming αCGRP are distributed among autonomic fibers that innervate the media of the vasculature (12). The location of these nerve endings as well as the potent vasodilatory effects of αCGRP (13) support the concept that αCGRP serves as a modulator of peripheral vascular tone. Importantly, there is evidence that CGRP colocalizes with nitric oxide (NO)-synthase in perivascular NANC nerves of various vascular beds (14).

Molecular Mechanisms Underlying CGRP Release

The mechanism leading to CGRP release, however, is still under debate. As mentioned above, in the cerebral feline circulation the vasodilator response to GTN and sodium nitroprusside, but not that to adenosine, is attenuated by the inhibitor CGRP_{8-37} (6), suggesting that CGRP release is caused by both nitrovasodilators. In the rat aorta, relaxations to GTN, but not sodium nitroprusside, are affected by CGRP_{8-37} (7,15). Whether NO directly or its second messenger cGMP indirectly stimulates the release of CGRP

*Editorials published in the *Journal of the American College of Cardiology* reflect the views of the authors and do not necessarily represent the views of JACC or the American College of Cardiology.

From the Medizinische Klinik, Johannes Gutenberg Universität, Mainz, Germany.



remains contradictory. An inhibitor of guanylate cyclase, methylene blue, attenuated CGRP release in rat femoral vessels by GTN (9), but not capsaicin-evoked CGRP release in the spinal cord (16). The latter showed pronounced modulation by NO but not by cGMP levels (16,17). Furthermore, the platelet inhibitory effects of CGRP in response to GTN were inhibited by N^G -monomethyl-L-arginine, a NO synthase inhibitor, and by the deletion of the endothelial NO synthase gene, suggesting that GTN antiplatelet activity of CGRP is mediated primarily through the activation of eNOS (10).

In this issue of the *Journal*, Guo et al. (18) report for the first time that CGRP partially mediates nitroglycerin effects in humans through an ALDH-2-dependent mechanism. The investigators show that acute GTN challenges cause systolic blood pressure decreases and increases in heart rate that correlate well with increased CGRP levels in plasma. In addition, in vitro incubation of cultured peripheral blood mononuclear cells with GTN increased CGRP mRNA expression. The increase in CGRP levels was markedly less pronounced in cells from individuals with a functional loss of ALDH-2 activity because of a Glu504Lys polymorphism

of the enzyme or in response to its inhibition by chloral hydrate.

The present studies clearly extend the latest findings about GTN metabolism by ALDH-2 (19) and show for the first time a contribution of ALDH-2 in the GTN-induced CGRP release in humans. The involvement of the ALDH-2 in GTN-induced effects on hemodynamics such as blood pressure, heart rate, plasma CGRP level are well documented.

Despite these achievements, several additional issues need to be addressed in the future. The degree of the contribution of CGRP to GTN-induced vasodilation remains uncertain. This issue could be solved by the use of specific CGRP inhibitors, whenever they will be available for human use. Other important questions are whether all organic nitrates (in particular mononitrates and dinitrates) and/or other nitrovasodilators, such as sodium nitroprusside, cause vasodilation in humans by increasing CGRP levels, and whether this effect is mediated by NO or cGMP. It would be also important to study whether a blunting of CGRP release during chronic treatment with GTN contributes to the development of nitrate tolerance.

GTN Tolerance and CGRP

Another important issue that needs to be addressed is the question of whether sustained GTN treatment may limit the potency of GTN to stimulate CGRP release. In 2005, Chen et al. (20) reported that GTN biotransformation and the activity of the GTN-activating enzyme ALDH-2 were decreased in GTN-tolerant vessels, pointing to a crucial role of ALDH-2 in GTN tolerance.

These observations were extended by our group in an animal model of in vivo tolerance (21) and in response to long-term clinical GTN application in patients (19) by showing that reactive oxygen species arising from mitochondria inhibited ALDH-2 activity and subsequently blocked the GTN biotransformation process associated with tolerance development. Biochemically, GTN-triggered reactive oxygen species formation caused an oxidation of sulfhydryl groups at the active site and consequently an inhibition of the enzyme (Fig. 1). The activity of ALDH-2 can be restored by sulfhydryl-group donors (19,22) and antioxidants such as reduced lipoic acid (22). Interestingly, recent experimental data indicate that decreased CGRP release accounts for in vivo GTN tolerance and that tolerance, as well as CGRP release, can be restored by treatment with sulfhydryl donors, such as N-acetylcysteine and captopril (8). On the other hand, development of in vitro GTN tolerance in rat aortic rings was reported to be counteracted by increased CGRP release (23), giving room for further studies to come.

In conclusion, the findings of Guo et al. (18) further strengthen the functionally relevant role of ALDH-2 in GTN bioactivation in humans. Their findings also indicate that the neuropeptide CGRP partially mediates GTN-

induced vasodilation, compatible with the concept that GTN indeed hits the nerve. The mechanistic involvement of CGRP in GTN-induced vasodilation as well as its impact on nitrate tolerance phenomena in humans needs further investigation.

Reprint requests and correspondence: Dr. Ulrich Hink, II. Medizinische Klinik, Johannes Gutenberg Universität, Langenbeckstrasse 1, 55131 Mainz, Germany. E-mail: hink@uni-mainz.de.

REFERENCES

- Nakamura Y, Moss AJ, Brown MW, Kinoshita M, Kawai C. Long-term nitrate use may be deleterious in ischemic heart disease: a study using the databases from two large-scale postinfarction studies. Multicenter Myocardial Ischemia Research Group. *Am Heart J* 1999;138:577–85.
- Chen Z, Zhang J, Stamler JS. Identification of the enzymatic mechanism of nitroglycerin bioactivation. *Proc Natl Acad Sci U S A* 2002;99:8306–11.
- Munzel T, Daiber A, Mulsch A. Explaining the phenomenon of nitrate tolerance. *Circ Res* 2005;97:618–28.
- Munzel T, Feil R, Mulsch A, Lohmann SM, Hofmann F, Walter U. Physiology and pathophysiology of vascular signaling controlled by guanosine 3',5'-cyclic monophosphate-dependent protein kinase [corrected]. *Circulation* 2003;108:2172–83.
- Mackenzie IS, Maki-Petaja KM, McEniery CM, et al. Aldehyde dehydrogenase 2 plays a role in the bioactivation of nitroglycerin in humans. *Arterioscler Thromb Vasc Biol* 2005;25:1891–5.
- Wei EP, Moskowitz MA, Boccalini P, Kontos HA. Calcitonin gene-related peptide mediates nitroglycerin and sodium nitroprusside-induced vasodilation in feline cerebral arterioles. *Circ Res* 1992;70:1313–9.
- Booth BP, Tabrizi-Fard MA, Fung H. Calcitonin gene-related peptide-dependent vascular relaxation of rat aorta. An additional mechanism for nitroglycerin. *Biochem Pharmacol* 2000;59:1603–9.
- Zhou ZH, Jiang JL, Peng J, Deng HW, Li YJ. Reversal of tolerance to nitroglycerin with N-acetylcysteine or captopril: a role of calcitonin gene-related peptide. *Eur J Pharmacol* 2002;439:129–34.
- Zhou ZH, Deng HW, Li YJ. The depressor effect of nitroglycerin is mediated by calcitonin gene-related peptide. *Life Sci* 2001;69:1313–20.
- Lee WI, Xu Y, Fung SM, Fung HL. eNOS-dependent vascular interaction between nitric oxide and calcitonin gene-related peptide in mice: gender selectivity and effects on blood aggregation. *Regul Pept* 2003;110:115–22.
- Bell D, McDermott BJ. Calcitonin gene-related peptide in the cardiovascular system: characterization of receptor populations and their (patho)physiological significance. *Pharmacol Rev* 1996;48:253–88.
- Holzer P. Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. *Neuroscience* 1988;24:739–68.
- Brain SD, Williams TJ, Tippins JR, Morris HR, MacIntyre I. Calcitonin gene-related peptide is a potent vasodilator. *Nature* 1985;313:54–6.
- Edvinsson L, Mulder H, Goadsby PJ, Uddman R. Calcitonin gene-related peptide and nitric oxide in the trigeminal ganglion: cerebral vasodilatation from trigeminal nerve stimulation involves mainly calcitonin gene-related peptide. *J Auton Nerv Syst* 1998;70:15–22.
- Peng J, Li YJ. New insights into nitroglycerin effects and tolerance: role of calcitonin gene-related peptide. *Eur J Pharmacol* 2008;586:9–13.
- Garry MG, Walton LP, Davis MA. Capsaicin-evoked release of immunoreactive calcitonin gene-related peptide from the spinal cord is mediated by nitric oxide but not by cyclic GMP. *Brain Res* 2000;861:208–19.
- Hughes SR, Brain SD. Nitric oxide-dependent release of vasodilator quantities of calcitonin gene-related peptide from capsaicin-sensitive nerves in rabbit skin. *Br J Pharmacol* 1994;111:425–30.
- Guo R, Chen X-P, Guo X, et al. Evidence for involvement of calcitonin gene-related peptide in nitroglycerin response and association with mitochondrial aldehyde dehydrogenase-2 (ALDH2) Glu504Lys polymorphism. *J Am Coll Cardiol* 2008;52:953–60.
- Hink U, Daiber A, Kayhan N, et al. Oxidative inhibition of the mitochondrial aldehyde dehydrogenase promotes nitroglycerin tolerance in human blood vessels. *J Am Coll Cardiol* 2007;50:2226–32.
- Chen Z, Foster MW, Zhang J, et al. An essential role for mitochondrial aldehyde dehydrogenase in nitroglycerin bioactivation. *Proc Natl Acad Sci U S A* 2005;102:12159–64.
- Sydow K, Daiber A, Oelze M, et al. Central role of mitochondrial aldehyde dehydrogenase and reactive oxygen species in nitroglycerin tolerance and cross-tolerance. *J Clin Invest* 2004;113:482–9.
- Wenzel P, Hink U, Oelze M, et al. Role of reduced lipoic acid in the redox regulation of mitochondrial aldehyde dehydrogenase (ALDH-2) activity. Implications for mitochondrial oxidative stress and nitrate tolerance. *J Biol Chem* 2007;282:792–9.
- Ghatta S, O'Rourke ST. Nitroglycerin-induced release of calcitonin gene-related peptide from sensory nerves attenuates the development of nitrate tolerance. *J Cardiovasc Pharmacol* 2006;47:175–81.

Key Words: nitroglycerin ■ mitochondrial aldehyde dehydrogenase ■ nitrate tolerance ■ calcitonin gene-related peptide.